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Influence of DNA, Alginate, Lysozyme and Bovine Serum Albumin on Sodium Silicate Condensation

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ABSTRACT

The interaction of DNA, alginate, Lysozyme and Bovine Serum Albumin with diluted solutions of sodium silicate was studied using the molybdosilicate method. DNA and alginate showed very weak interactions with silica precursors whereas both proteins were able to form silica gels. Both electrostatic interactions and hydrogen bonds are suggested to arise between peptide chain and polysilicates, bringing new informations on the nature of inorganic and bio-organic species involved in the natural biosilicification processes.

INTRODUCTION

Biomineralization processes often occur at the interface between inorganic precursors and biological macromolecules [1]. For instance, bone formation involves hydroxyapatite deposition in a collagen proteinaceous matrix whereas the β-chitin polysaccharide and calcium carbonate are associated within crab cuticles. The formation of the silica skeleton of diatoms was shown to take place mainly at the interface with proteins, even though the presence of sugars in the cell wall was reported [2-3].

Aiming at understanding the interactions that may arise between the naturally-occurring form of silica precursors, i.e. silicic acids, and proteins, we have undertaken the study of the effect of various biopolymers on the behaviour of sodium silicate diluted solutions. We have first focused on amino acids and poly-amino acids and shown that poly-lysine and poly-arginine were able to induce silica formation, the catalytic effect increasing with polymer chain length [4-5]. These results correlate well with the fact that the silaffin proteins that were extracted from diatom cells are characterized by lysine and arginine patches along the peptide chain [6].

In order to get a better understanding of the possible silica-biopolymers interactions, we have selected two proteins that bear an important number of lysine and arginine groups, as well as a polysaccharide macromolecule and DNA. The evolution of the silicic acid content of a diluted sodium silicate solution in the presence of these polymers was monitored using the molybdosilicate method [7]. Depending on the solution pH, only the two proteins were able to induce silica formation. These results are discussed in terms of electrostatic interactions and hydrogen bonds.

EXPERIMENTAL

Waterglass (27% SiO_2, 10 % NaOH) from Riedel-de Haën was chosen as the source of silicic acid. Aqueous silicate solutions were preferred to silicon alkoxide precursors because they correspond to the usual form of soluble silica in nature. For similar reasons, diluted solutions (10 mM) of SiO_2 were used. Lysozyme grade I from Chicken Egg White and Bovine Serum Albumin (BSA) were purchased from Sigma. Deoxyribonucleic acid (DNA) from fish sperm was obtained from Amersham and alginic acid sodium salt from brown algae from Fluka. Molybdosilicate studies were performed in a 0.05 M Tris-HCl buffer (pH = 7.2) and 0.05 M acetate buffer (pH = 4.9).

In a typical experiment, 65 mg of the sodium silicate solution were diluted in 30 mL of the appropriate buffer in order to obtain a 10^{-2} M silica solution. The mixture was stirred for ten minutes before adding a solution containing the appropriate quantity of polymer so that the monomer (i.e amino acid, sugar or DNA unit)-to-Si ratio was kept equal to 0.1 dissolved in 3 mL of the buffer solution. At regular intervals of time, 400 µl of the reacting solution were taken and added to 5 ml of deionized water. 200 µl of H_2SO_4 (1.5 M) and 200 µl of ammonium molybdate (0.08 M) were then added [8]. The mixture was left to stand for 10 minutes in order to allow monomeric silicic acid and small silica oligomers to react with the heptamolybdic acid to form the yellow silicomolybdic acid $H_8Si(Mo_2O_7)_6$. The optical density (OD) of the final solution was measured at 400 nm using a double beam Uvikon XS spectrophotometer. Data were reproducible within a 5 % error range.

When silica formation occurred, solids were centrifuged and freeze dried at – 30°C. Thermogravimetric analysis were performed on a Netzch STA409 apparatus under O_2 flow with a heating rate of 5°C/min.

BSA and Lysozyme titration curves were calculated using ExPASY softwares from the Swiss Institute of Bioinformatics (SIB) [9].

RESULTS

Silicic acid is obtained upon acidification and dilution of sodium silicate solutions. A clear solution (10 mM in silica) is obtained. In a Tris.HCl buffer (pH = 7.2), the initial amount of silicic acid decreases slowly but no gelation is observed within the first 3 hours (Figure 1). This amount decreases slightly faster in the presence of DNA, alginate and BSA but the solutions remain optically clear and gelation is not observed either. Addition of Lysozyme induces a first increase in silicic acid content followed by a slow decrease and solutions become turbid, leading to the formation of a loose gel.

In contrast, in acetate buffer (pH = 4.9), a slow increase of initial silicic acid content is observed that is only slightly modified by biopolymers addition (Figure 2). No gel is formed except in the presence of BSA.

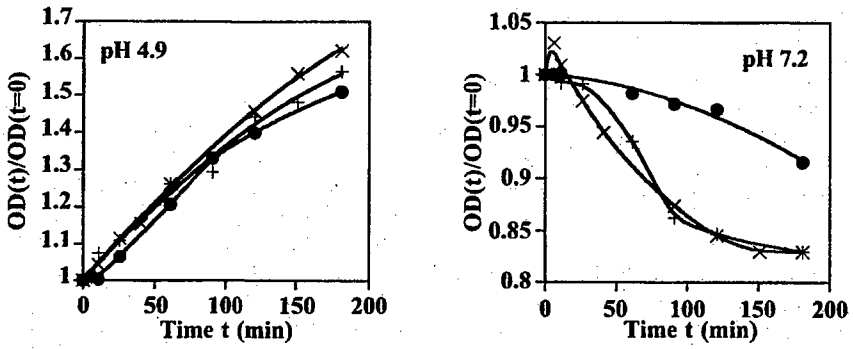

Figure 1 Condensation of silicic acid in the absence (●) and in the presence of DNA (+) and alginic acid (x) in acetate (left) and Tris.HCl (right) buffer. Evolution with time of the silicic acid content as measured by the optical density ratio OD(t)/OD(t=0) at 400 nm using the molybdosilicate method.

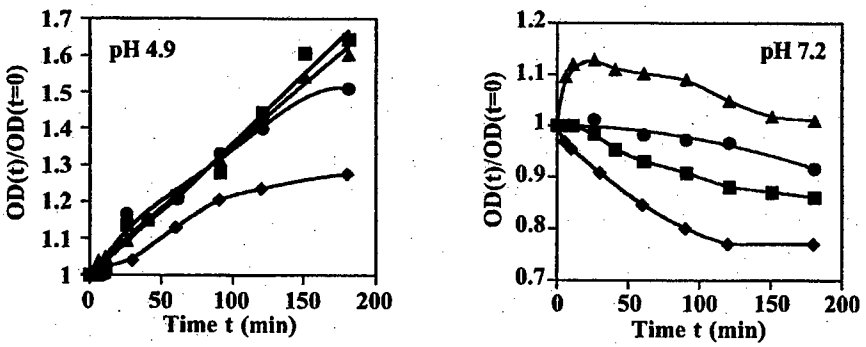

Figure 2 Condensation of silicic acid in the absence (●) and in the presence of Lysozyme (▲), BSA (■) and poly-Lysine (◆) in acetate (left) and Tris.HCl (right) buffer. Evolution with time of the silicic acid content as measured by the optical density ratio OD(t)/OD(t=0) at 400 nm using the molybdosilicate method.

After freeze drying, the two obtained solids, hereafter named Lyso7 and BSA4, contain *c.a.* 50 w% of silica. Corresponding yields, calculated on the basis of the initial solution molar Si content, are 20% for Lyso7 and 10 % for BSA4.

DISCUSSION

Concentrated sodium silicate solutions are known to contain a mixture of oligomeric silicate anions, ranging from chain trimers to cage octamers [10]. Upon dilution, decondensation occurs leading to monomolecular $[SiO_4H_{4-x}]^{x-}$ species, where x increases with pH above the point of zero charge (pH ≈ 3). $Si(OH)_4$ is the predominant species in the pH range 4-9 and $[SiO(OH)_3]^-$ above pH ≈ 10 [11]. However, monomeric silicic acid exists only in very dilute solutions. At higher concentration, polymerization occurs by condensation of silanol Si-OH groups between neutral silicic acid and negatively charged species. The rate of condensation goes through a minimum around the point of zero charge and increases with pH. It is for instance two orders of magnitude faster at pH 6 than at pH 4 [12]. Therefore, two opposite processes, decondensation and polymerization, compete when concentrated sodium silicate solutions are diluted. At pH 7.2, the condensation process is faster so that a small decrease in silicic acid content is observed whereas the decondensation process mainly predominate at pH 4.9.

In the 4.5-7.5, pH domain, both DNA and alginic acid are negatively charged so that only repulsive interactions are expected to occur with negatively charged silica oligomers. However, the slight decrease in the silicic acid content when compared to sodium silicate alone at both pH suggest that monomeric species may weakly interact through hydrogen bonding with the polymers.

In contrast, the observed formation of Lyso7 and BSA4 is rather surprising when compared to previous studies on p-Lysine [5]. In the same experimental conditions, addition of this peptide led to rapid silica polymerization at pH 7 and not at pH 4, close to Lysozyme behaviour. However, as shown on Fig.1 and Fig.2, silica precipitation was correlated to an important decrease in silicic acid content at pH 7. This was attributed to the possibility for monomeric silica to interact electrostatically with lysine amine group and through hydrogen bonds with peptide backbone carbonyls. Because lysine groups are present all along the peptide chain, precursors are brought close enough to promote condensation [5]. In the case of the two proteins used in this work however, lysine and arginine groups are distributed along the peptide chain so that silica monomers may be too far from one another to interact. This argument, combined with the absence of important decrease in silicic acid content, suggests that the silica species responsible for gel formation are not, in this case, monomers but correspond to the oligomeric anions present in the starting solution. This hypothesis is strengthened by the fact that only a small part (10-20 %) of the initial silica is present in the final solids, indicating that participation of monomers through condensation is limited.

The difference in behaviour of Lysozyme and BSA in acidic and neutral media can be correlated to both silica species and proteins charges. Calculated titration curves for both proteins are given in Fig. 3. BSA is a large protein (MW ≈ 66 kDa) containing 582 amino acid residues. Basic lysine and arginine groups account for *c.a.* 14 % of the total sequence whereas acidic aspartatic acid and glutamic acid account for *c.a.* 16 %. As a consequence, its point of zero charge is close to pH 5. In contrast, Lysozyme is a small chain peptide (MW ≈ 16 kDa, 147 amino acids) with 12 % basic and 6% acidic groups with a point of zero charge close to 11. Lysozyme bears a small nearly constant (*c.a.* 10-12) positive charge in the 4.5-7.5 pH range. In contrast, BSA is slightly negatively charged at pH 7.2 and positively charged at pH 4.9.

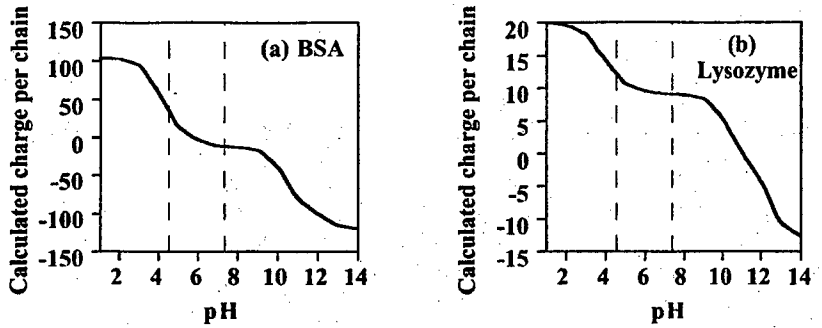

Figure 3 Evolution of calculated charge per chain with pH for (a) BSA and (b) Lysozyme.

Therefore, at neutral pH, BSA and silicate bear charges of same sign and no interaction is expected whereas electrostatic interactions may arise between positively charged Lysozyme and anionic silicates, thus explaining the observed silica gelation. Accordingly, at pH 4.9, BSA becomes positively charged leading to BSA4 formation. However, at this pH, it should be expected that Lysozyme also induces silica formation, in contradiction with experimental results. Since Lysozyme charge is only slightly modified by the pH decrease, the explanation should lie in the silicate reactivity. Even though it is difficult, at this time, to conclude, it may be attributed to the decrease of polysilicates negative charges as pH comes closer to silica point of zero charge around pH 3.

CONCLUSION

These studies aimed at identifying the nature of inorganic and bio-organic species, which are likely to be involved in biosilicification processes. Considering activation of silica polymerisation, this work suggests that proteins are the most likely to interact with mineral precursors. However, it should be noticed that polymers like polysaccharides might be involved in other aspects of silica biomineralization such as morphology control, as previously suggested [3, 13]. As far as silica precursors are concerned, our results suggest that oligomeric polysilicates may be involved in the silicification process. These species therefore add to monomeric silica and silica nanoparticles which were previously shown to form silica gels in the presence of poly-amino acids [5]. Such diversity reflects the complexity of natural processes that is the main challenge of the biomimetic design of new materials.

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